IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:
DERAND et al. : GROUP ART UNIT: 1636

SERIAL NO: 10/665,283

FILED: 09/22/2003 : EXAMINER: Jennifer Ann DUNSTON

FOR: « ION CHANNEL HYBRID PROTEINS AS ELECTRICAL SENSORS AND USE THEREOF. »

DECLARATION UNDER 37 C.F.R. 1.132

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C.

SIR:

Now comes Michel VIVAUDOU, who declares and states that:

1. I am a research scientist, group leader in the laboratory of Molecular and Cellular Biophysics at the CEA research center in Grenoble, France. I graduated from the french engineering school 'Ecole Centrale de Lyon', obtained a MS in electrical engineering, a MS in biomedical engineering and a PhD in Biomedical Engineering at the Worcester Polytechnic Institute in Worcester, Mass., USA.

2. I have been employed by COMMISSARIAT A L'ENERGIE ATOMIQUE since 1988.

3. I declare that I am experienced in the field of ion channel proteins as it emerges from the brief curriculum vitae and the publication list herewith attached.

4. I hereby declare that I had full success in constructing and expressing fusion proteins based on numerous ABC transporters (MRP1, YCF1, MDR1...). The techniques involved are straightforward for the skilled artisan. The obtained results are exposed in herewith attached figures 1 and 2.

Figure 1 represents the effects of MDR1 substrates on channel activity of fusion protein MDR1-Kir6.2 measured in *Xenopus* oocyte excised patches (see method, page 17 of the instant specification). Comparison with the construct MDR1 (TMD0SUR2A)+Kir6.2 where the N-terminal domain TMD0 of SUR2A, which has been identified as the domain interacting with Kir6.2, is fused at the N-terminal end of MDR1. The latter construct assembles spontaneously with Kir6.2. Both constructs are significantly inhibited by the tested compounds.

Figure 2 shows the effect of glibenclamide, a known substrate of MRP1, on channel activity of the fusion protein MRP1-Kir6.2. The trace shown is a patch clamp record measured in *Xenopus* oocyte in the excised inside-out configuration. Hybrid channels are significantly inhibited by the tested compound.

Thus any ABC transporter coupled to Kir6.2 or in some cases coupled to a slightly modified Kir6.2 protein solves effectively the instant problem.

Indeed, considering the herewith attached figure 1 and the data provided in the specification, MRP1, MDR1 and YCF1 coupled to Kir6.2 or Kir6,2ΔC36, form functional channels and are able to be used as electrical sensors.

As specified here above, figure 1 relates to MDR1 and shows evidence that MDR1-Kir6.2 hybrid protein is properly folded, trafficked and inserted into the membrane, such that a functional channel is formed. Such hybrid protein is well expressed and is modulated by several substrates of MDR1.

5, Aim of the instant invention

I hereby declare that it is true that nucleotide binding to MRP1 does not induce a change in Kir6.2 channel activity.

However, the aim of the instant invention is not to record the binding of nucleotides which target cytosolic domains of ABC transporters and are of little interest for screening, but the binding of substrates or modulators which are known to target the transmembrane domains of MRP1.

As shown, in the herewith attached figure 2, I have evidence that binding of substrate to MRP1 can indeed modulate channel activity measurably.

This is also clearly specified on page 2, lines 26-30 of the specification.

Therefore, constructs with MRP1 solve effectively the instant problem and Baukrowitz and al document is not pertinent.

7. I hereby declare that there is no need to have the knowledge of the mechanism of action if the product is characterized and if there exists tests in view to verify the properties of said product.

8. I declare that there is no undue experiments to perform in view to obtain the products of the instant invention; indeed, the man skilled in the art has all the means to effectively obtain the products of the invention without undue experiments:

- nucleic acid sequence encoding an ABC transporter: all the sequences are available in the data bases; further more there exist many publications giving the predicted secondary structure of ABC transporters. Eucaryotic ABC transporters are known to contain two cytosolic nucleotide binding domains and 2 or 3 transmembrane domains which are believed to form the transmembrane cavity required for the transport function of ABC proteins.
- nucleic acid sequence encoding Kir6.2 or a derivative thereof; Kir6.2 has been described, for instance by Inagaki et al, Science, 1995, 270, 1166-1170
- making a fusion construct with the ABC transporter; the specification provides all elements for making such a construct
- determining if the obtained hybrid protein is capable of functioning as a biosensor; the specification provides all elements for making such a construct; further more the instant figures 1 and 2 confirm the pertinence of the selected tests

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the Application or any Patent issued thereon.

September 29, 2005
Date

(signature)

Michel VIVAUDOU

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Born on January,10 1957 in Salindres, France
French citizenship, married, two children

Degrees

1979: B.S. Electrical Engineering - Ecole Centrale, Lyons, France
1979: D.E.A. Solid-State Electronics - Ecole Centrale, Lyons, France
1980: M.S. Biomedical Engineering - Worcester Polytechnic Institute, MA, USA
1984: M.S. Electrical Engineering - Worcester Polytechnic Institute, MA, USA
1984: Ph.D. Biomedical Engineering - Worcester Polytechnic Institute, MA, USA

Research Experience

1979-80: Graduate student (Rhône-Poulenc fellowship), Biomedical Engineering Department, Worcester Polytechnic Institute, Worcester, Mass. USA
Design of a single thermistor system for continuous cardiac output measurement

1982-84: Research Assistant, Biomedical Engineering Department, Worcester Polytechnic Institute
Analysis of pilot eye movement to optimize cockpit design and assess workload
Design of a communication interface controlled by saccadic eye movement for the handicapped

1984-86: Postdoctoral Research Associate, Department of Physiology, University of Massachusetts Medical School, Worcester, Mass. USA
Computer analysis of single-channel records of Ca^{2+}-activated K^+ channels from smooth muscle
Hormonal and neurotransmitter regulation of voltage-activated Ca^{2+} currents in smooth muscle

1986-88: Instructor, Department of Physiology, University of Massachusetts Medical School
Properties and regulation of whole-cell and single-channel Ca^{2+} currents in smooth muscle

1988-90: Postdoctoral fellow, Laboratoire de Biophysique Moléculaire et Cellulaire, CENG, Grenoble, France
Identification and nucleotide regulation of ATP-sensitive K^+ channels in frog skeletal muscle

1990-95: Tenured investigator, Laboratoire de Biophysique Moléculaire et Cellulaire, CENG
Physiological and pharmacological regulation of skeletal muscle ATP-sensitive K^+ channels

1995-96: Associate Professor, Department of Physiology and Biophysics, Mount Sinaï School of Medicine, New York.
Structure and function of G-protein gated K^+ Channels

1996-++: Principal Investigator, Group Leader, Laboratoire de Biophysique Moléculaire et Cellulaire, CEA/Grenoble
Molecular pharmacology of ion channels and ABC transporters

Languages

French (native), English (fluent), German (average)

Publications

[1] Tole JR, Vivaudou M, Harris RL, Ephrath A. (1982) FMP study of pilot workload. Qualification of workload via instrument scan. *NASA Report CR-169254.* 6pp.

[2] Tole JR, Vivaudou M, Stephens AT, Harris RL, Ephrath A. (1982) Entropy, instrument scan, and pilot workload. *NASA Report TM-85145.* 8pp.

[3] Tole JR, Stephens AT, Vivaudou M, Ephrath A, Young LR. (1983) Visual scanning behavior and pilot workload. *NASA Report CR-3717.* 41pp.

[4] Vivaudou MB, Singer JJ, Walsh, JV Jr. (1986) An automated technique for analysis of current transitions in multilevel single-channel recordings. *Pflügers Archiv.* **407**:355-364

[5] Clapp LH, Vivaudou MB, Walsh JV Jr, Singer JJ. (1987) Acetylcholine increases voltage-activated Ca^{2+} current in freshly dissociated smooth muscle cells. *Proc. Natl. Acad. Sci. USA.* **84**:2092-2096

[6] Vivaudou MB, Clapp LH, Walsh JV Jr., Singer JJ. (1988) Regulation of one type of Ca^{2+} current by diacylglycerol and acetylcholine in smooth muscle cells. *FASEB J.* **2**:2497-2504

[7] Sims SM, Vivaudou MB, Clapp LH, Lassignal NL, Singer JJ, Walsh JV Jr. (1988) Neurotransmitter regulation of ionic channels in freshly dissociated smooth muscle cells. *Ann. N.Y. Acad. Sci.* **27**:346-359

[8] Clapp LH, Vivaudou MB, Singer JJ, Walsh JV Jr. (1989) Substance P, like acetylcholine, augments one type of Ca^{2+} current in isolated smooth muscle cells. *Pflügers Archiv.* **413**:565-567

[9] Sims SM, Vivaudou MB, Hillemeier C, Biancani P, Singer JJ., Walsh JV Jr. (1990) Membrane currents and cholinergic regulation of K^+ currents in esophageal smooth muscle cells. *Am. J. Physiol.* **258**:G794-G802

[10] Vivaudou MB, Singer JJ, Walsh JV Jr. (1991) Multiple types of Ca^{2+} channels in visceral smooth muscle cells. *Pflügers Archiv.* **418**:144-152

[11] Vivaudou MB, Arnoult C, Villaz M. (1991) Skeletal muscle ATP-sensitive K^+ channels recorded from sarcolemmal blebs of split fibers: ATP inhibition is reduced by Mg^{2+} and ADP. *J. Memb. Biol.* **122**:165-175

[12] Forestier C, Vivaudou M. (1993) Modulation by Mg^{2+} and ADP of ATP-sensitive K^+ channels in frog skeletal muscle. *J. Memb. Biol.*. **132**:87-94

[13] Forestier C, Depresle Y, Vivaudou M. (1993) Intracellular protons control the affinity of skeletal muscle ATP-sensitive K^+ channels for Potassium-Channel-Openers. *FEBS Lett.* **325**:276-280

[14] Vivaudou M, Forestier C. (1995) Modification by protons of frog skeletal muscle K_{ATP} channels: Effects on ion conduction and nucleotide inhibition. *J. Physiol-London.* **486**:629-645

[15] Allard B, Fournet G, Rougier O, Descans B, Vivaudou M. (1995) Dose-dependent activation and block by bisG10, a K^+ channel blocker, of mouse and frog skeletal muscle K_{ATP} channels. *FEBS Lett.* **375**: 215-219

[16] Forestier C, Pierrard J, Vivaudou M. (1996) Mechanism of action of K-Channel-Openers on frog skeletal muscle K_{ATP} channels: Interactions with nucleotides and protons *J. Gen. Physiol.* **107**:489-502

[17] Chan KW, Sui JL, Vivaudou M, Logothetis D. (1996) Control of G-protein-gated K^+ channel activity through a unique amino acid residue of the GIRK1 subunit. *Proc. Natl. Acad. Sci. USA.* **93**:14193-14198

[18] Chan KW, Sui JL, Vivaudou M, Logothetis DE. (1997) Specific Regions of Heteromeric Subunits Involved in Enhancement of G Protein-gated K^+ Channel Activity. *J. Biol. Chem.* **272**: 6548-6555

[19] Vivaudou M, Chan KW, Sui JL, Jan LY, Reuveny E, Logothetis DE. (1997) Probing the G-protein regulation of GIRK1 and GIRK4, the two subunits of the K_{ACh} channel, using functional homomeric mutants. *J. Biol. Chem.* **272**:31553-31560

[20] D'hahan N, Jacquet H, Moreau C, Catty P, Vivaudou M. (1999) A Transmembrane Domain of the Sulfonylurea Receptor Mediates Activation of K_{ATP} Channels by K-Channel-Openers. *Mol. Pharmacol.* **56**:308-315

[21] Sui JL, Chan KW., Langan MN, Vivaudou M, Logothetis DE. (1999) G Protein-Gated Potassium Channels. *Adv. Second Messenger Phosphoprotein Res.* **33**:179-201

[22] D'hahan N*, Moreau C*, Prost AL, Jacquet H, Alekseev AE, Terzic A, Vivaudou M (1999) Pharmacological plasticity of cardiac ATP-sensitive potassium channels toward diazoxide revealed by ADP. *Proc. Natl. Acad. Sci. USA.* **96**: 12162-12167

[23] Bienengraeber M, Alekseev AE, Abraham MR, Carrasco AJ, Moreau C, Vivaudou M, Dzeja PP, Terzic A (2000) ATPase activity of SUR2A: A catalytic function for the K_{ATP} channel complex. *FASEB J.* **14**:1943-1952

[24] Moreau C* , Jacquet H*, Prost AL, D'hahan N, Vivaudou M (2000) The molecular basis of the specificity of action of K_{ATP} channel openers. *EMBO J.* **19**:6644-51

[25] Terzic A, Vivaudou M (2001) Molecular pharmacology of ATP-sensitive K^+ channels: How and why? In: *Potassium channels in Cardiovascular Biology, Ed. S. Archer and N. Rusch, Kluwer/Plenum*, pp257-277

[26] Prost A, Dérand R, Gros L, Becq F, Vivaudou M (2003) Block of ATP-sensitive K^+ channels by substituted benzo[c]quinolizinium CFTR activators. *Biochem. Pharm.* **66**:425-30

[27] Prost A, Bloc A, Hussy N, Dérand R, Vivaudou M (2004) Zinc is both an intracellular and extracellular regulator of K_{ATP} channel function. *J. Physiol-London.* **559**:157-67

[28] Moreau C, Gally F, Jacquet-Bouix H, Vivaudou M (2005) The size of a single residue of the sulfonylurea receptor dictates the effectiveness of K_{ATP} channel openers. *Mol. Pharm.* **67**:1026-33

[29] Moreau C, Prost AL, Dérand R, Vivaudou M (2005) SUR, ABC proteins targeted by KATP channel openers. *J. Mol. Cell. Cardiol.* **38**:951-63

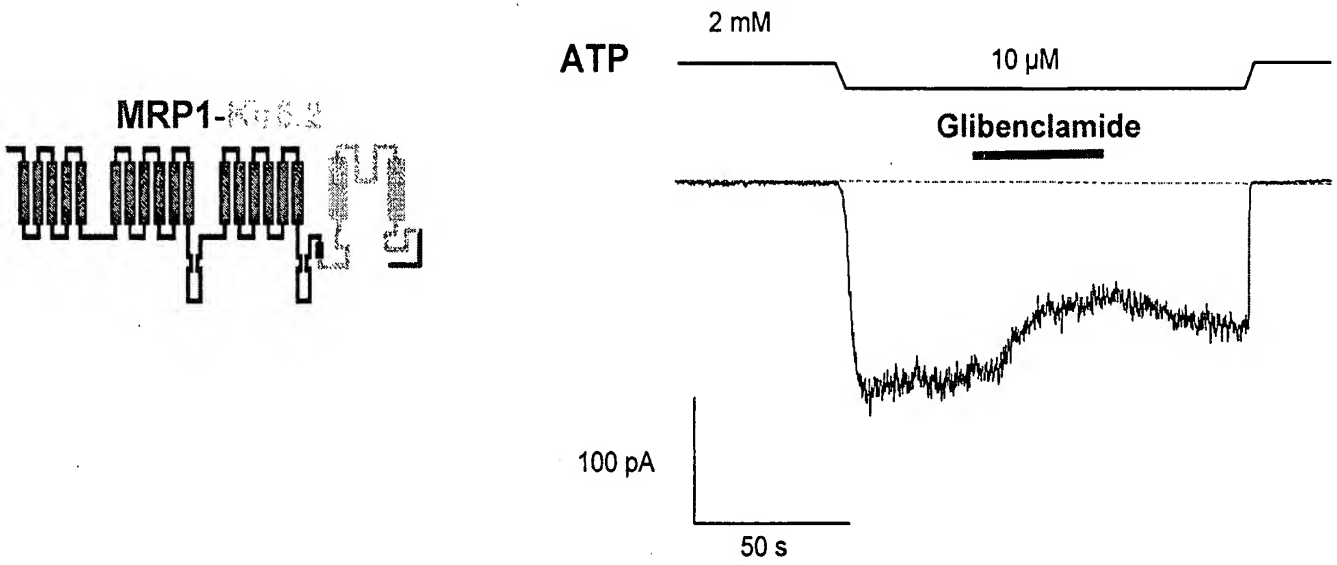

Fig.2. Effects of glibenclamide, a known substrate of MRP1 substrates on channel activity of fusion protein MRP1-Kir6.2. The trace shown is a patch clamp record measured in Xenopus oocyte in the excised inside-out configuration. Hybrid channels are significantly inhibited by the tested compound.

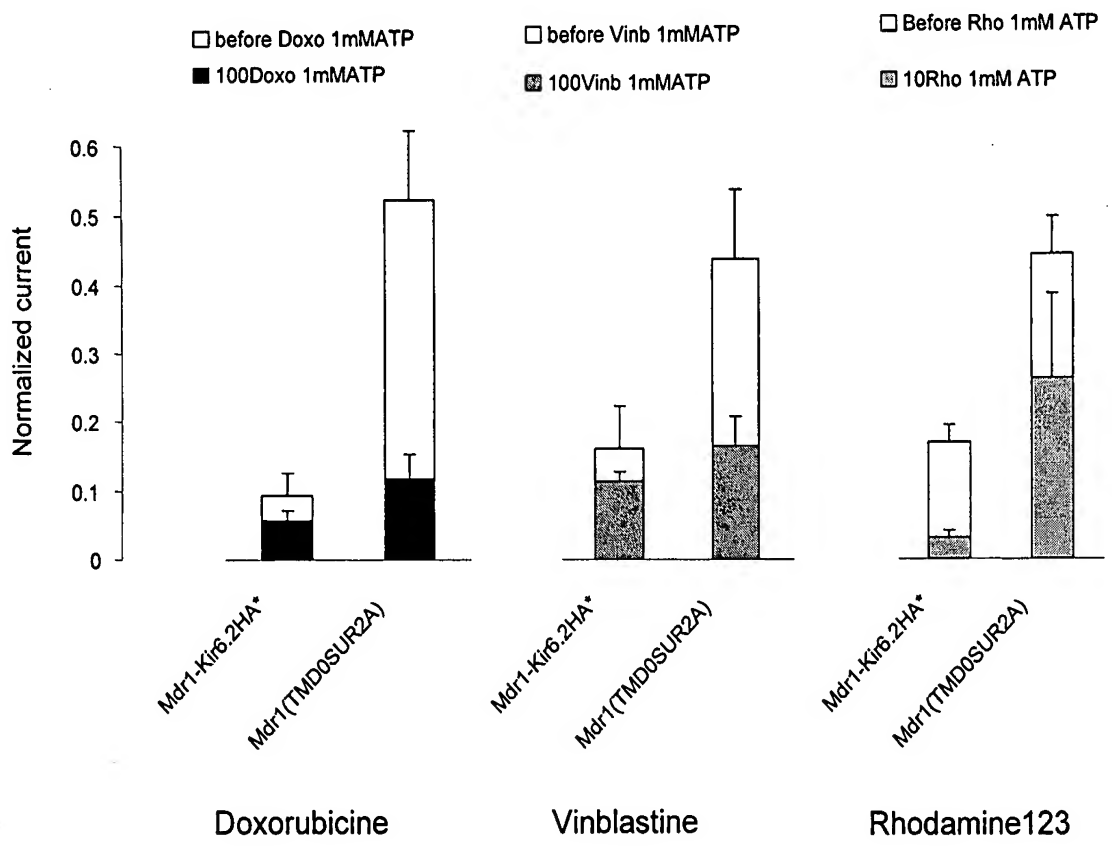

Fig.1. Effects of MDR1 substrates on channel activity of fusion protein MDR1-Kir6.2 measured in Xenopus oocyte excised patches. Comparison with the construct MDR1(TMD0SUR2A)+Kir6.2 where the N-terminal domain TMD0 of SUR2A, which we have identified as the domain interacting with Kir6.2, is fused at the N-terminal end of MDR1. The latter construct assembles spontaneously with Kir6.2. Both constructs are significantly inhibited by the tested compounds.